Hemophagocytic lymphohistiocytosis

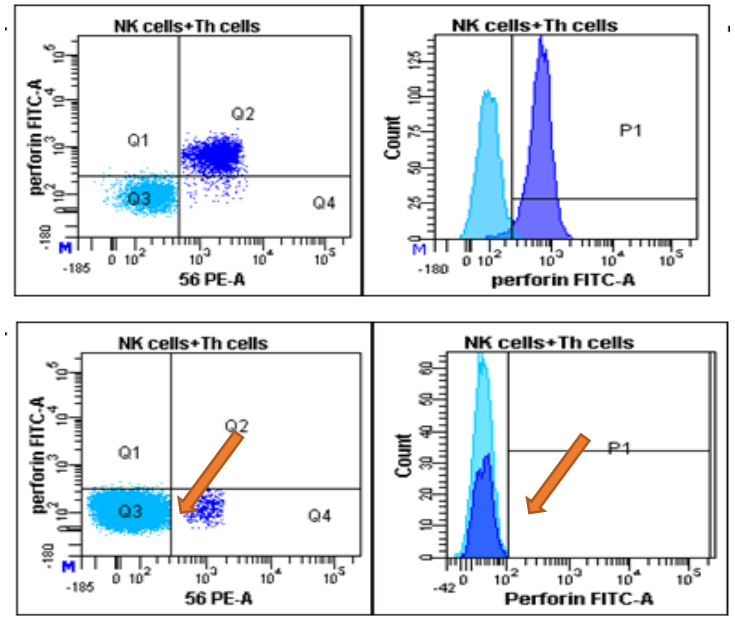
Case1

- 20 year old Male born with non consanguineous marriage and had no significant family history
- He was healthy till 18 years of age.
- He was diagnosed with abdominal tuberculosis at 18 years of age and was treated with anti-tuberculosis treatment for 18 months.
- His health improved and was clinically asymptomatic for 6 months and therefore anti-tuberculosis treatment was stopped.

- He was admitted with high-grade fever, pancytopenia (Hb: 8.2gh/dl, WBC count of 1600/mm3, platelet count of 25000/mm3) lymphadenopathy, and hepatosplenomegaly.
- His bone marrow aspirate revealed hemophagocytosis and was investigated further for HLH
- He also had elevated sFerritin levels
- No infections identified

HLH criteria		
Fever	Yes	3/4
Splenomegaly	Yes	
Cytopenia	Yes	
Hepatitis	No	
Hemophagocytosis	yes	4/4
Ferritin (>500mg/ml)	16,000ng/ml	
sCD25 levels (333-8750g/ml)	10455pg/ml	
NK cell function	Low	
Triglygerides (>265mg/dl)	811mg/dl	1/2
Fibrinogen (<150mg%)	216mg%	

- He was treated with HLH protocol 2004 including cyclosporine and dexamethasone. He responded well.
- He relapsed thrice while steroids were tapered. The patient was advised bone marrow transplantation.
- He was off therapy for more than 1 year and was asymptomatic.
- However, he had multiple relapses and expired due to severe disease.



***PRF1* gene mutation- compound heterozygous mutation c.386G>C p.Try129Ser and c.1471G>A p.Asp491Asn**

Hemophagocytic Lymphohistiocytosis-HLH

- HLH is a life-threatening condition of
  - severe hyperinflammation
  - caused by the uncontrolled proliferation of activated lymphocytes and macrophages secreting high amounts of inflammatory cytokines.
- Cardinal signs and symptoms are
  - prolonged fever
  - hepatosplenomegaly
  - pancytopenia.

HLH

- HLH is not a single disease
- It is a clinical syndrome that can be encountered in association with a variety of underlying conditions leading to the same characteristic hyperinflammatory phenotype
- This condition shares features with sepsis and systemic inflammatory response syndrome (SIRS)

Criteria for HLH diagnosis

- Prolonged fever (>7 days)
- Cytopenias affecting **two or three** of the three lineages in the peripheral blood:
  - Hemoglobin <90 g/L (for infants age <4 weeks: Hgb <100 g/L)
  - Platelets <100x109/L
  - Neutrophils <1.0x109/L
- Splenomegaly
- Hypertriglyceridemia and/or hypofibrinogenemia
  - Fasting triglycerides ≥2.0 mmol/L or >3 SD of the normal value for age
  - Fibrinogen ≤1.5 g/L

- Hemophagocytosis. Non-malignant, mixed lymphohistiocytic accumulation in the reticuloendothelial system; the spleen, liver, lymph nodes, bone marrow, and CNS are most frequently involved.
  - Note: (1) Hemophagocytosis may not be apparent early in the course of the disease. (2) Hemophagocytosis is seen less often in the liver, where lymphocytic infiltration of the portal areas is typical
- Low or absent natural killer (NK) cell activity. This is common prior to and during active disease, as well as after remission following chemotherapy in a significant proportion of individuals with FHL. Normal or raised NK cell activity has also been observed in some affected individuals, including those with *UNC13D* pathogenic variants
  - Note: (1) NK cell activity normative values are per local laboratory reference. (2) The number of circulating NK cells (CD56+/16+) is generally within normal limits. (3) In secondary (acquired or reactive) hemophagocytic lymphohistiocytosis (HLH), NK cell activity has been reported to normalize during

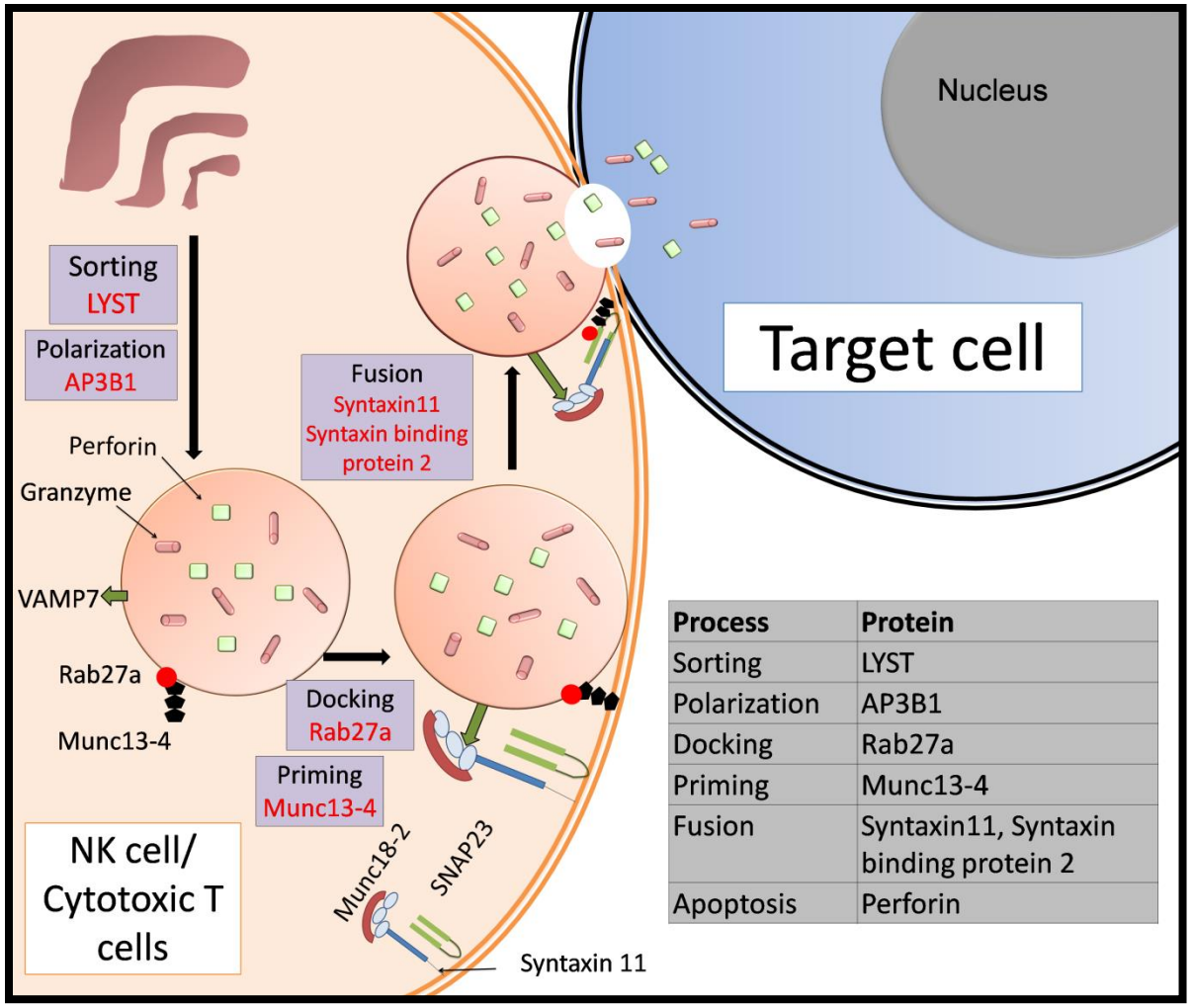
- Hyperferritinemia. Serum ferritin concentration ≥500 µg/L (normal 10-290 µg/L)
  - It is, however, markedly elevated in the majority of persons with HLH and is a very sensitive indicator of HLH when serum concentrations are markedly increased.
  - Increased serum ferritin concentrations make no distinction between genetic and secondary HLH.

- High plasma concentrations of soluble CD25 (soluble IL2Rα); ≥2400 U/mL
  - Note: (1) Normal range depends on test methodology used. (2) Results must be compared to age-matched controls.

Impaired NK cell function in HLH

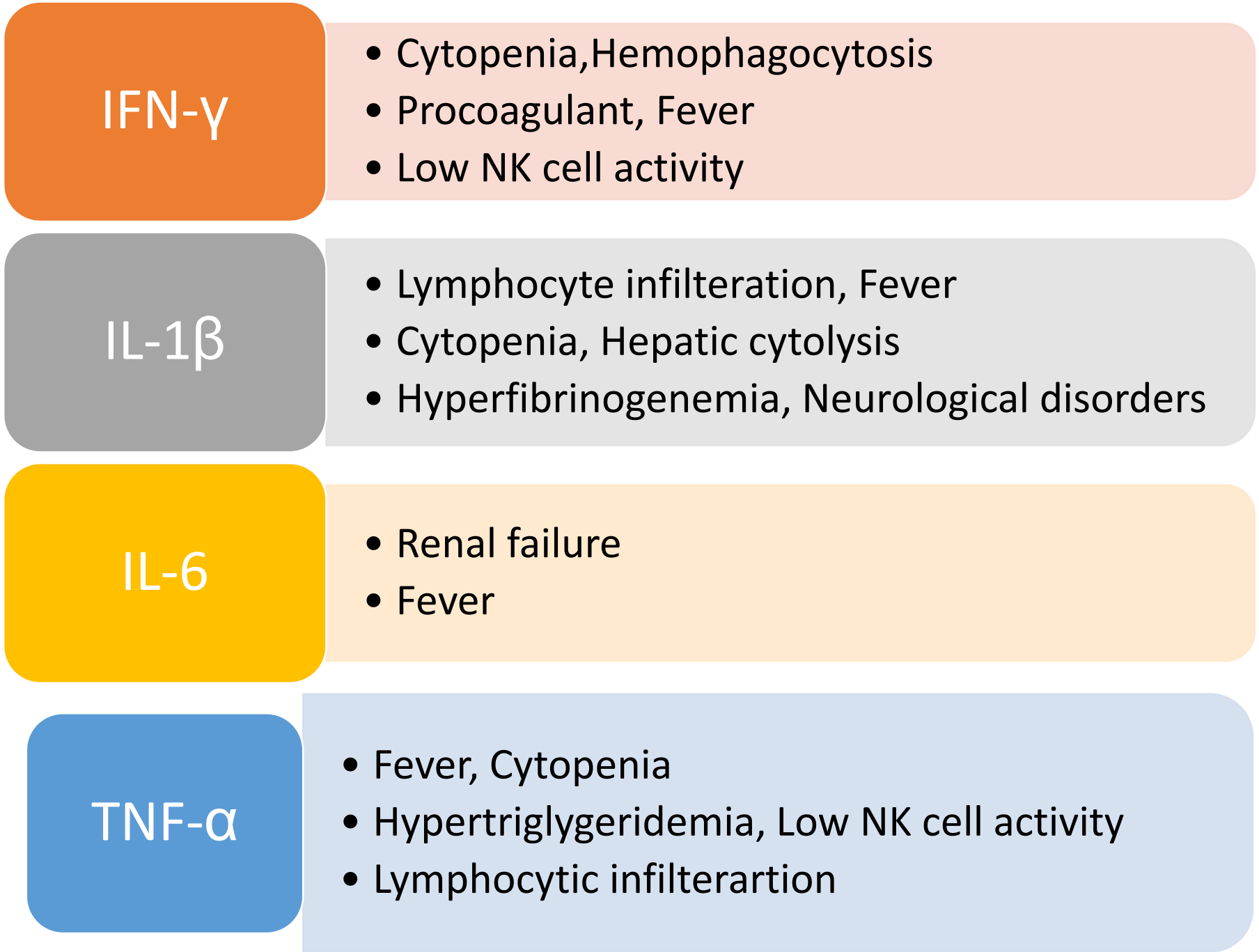
GENETIC HLH

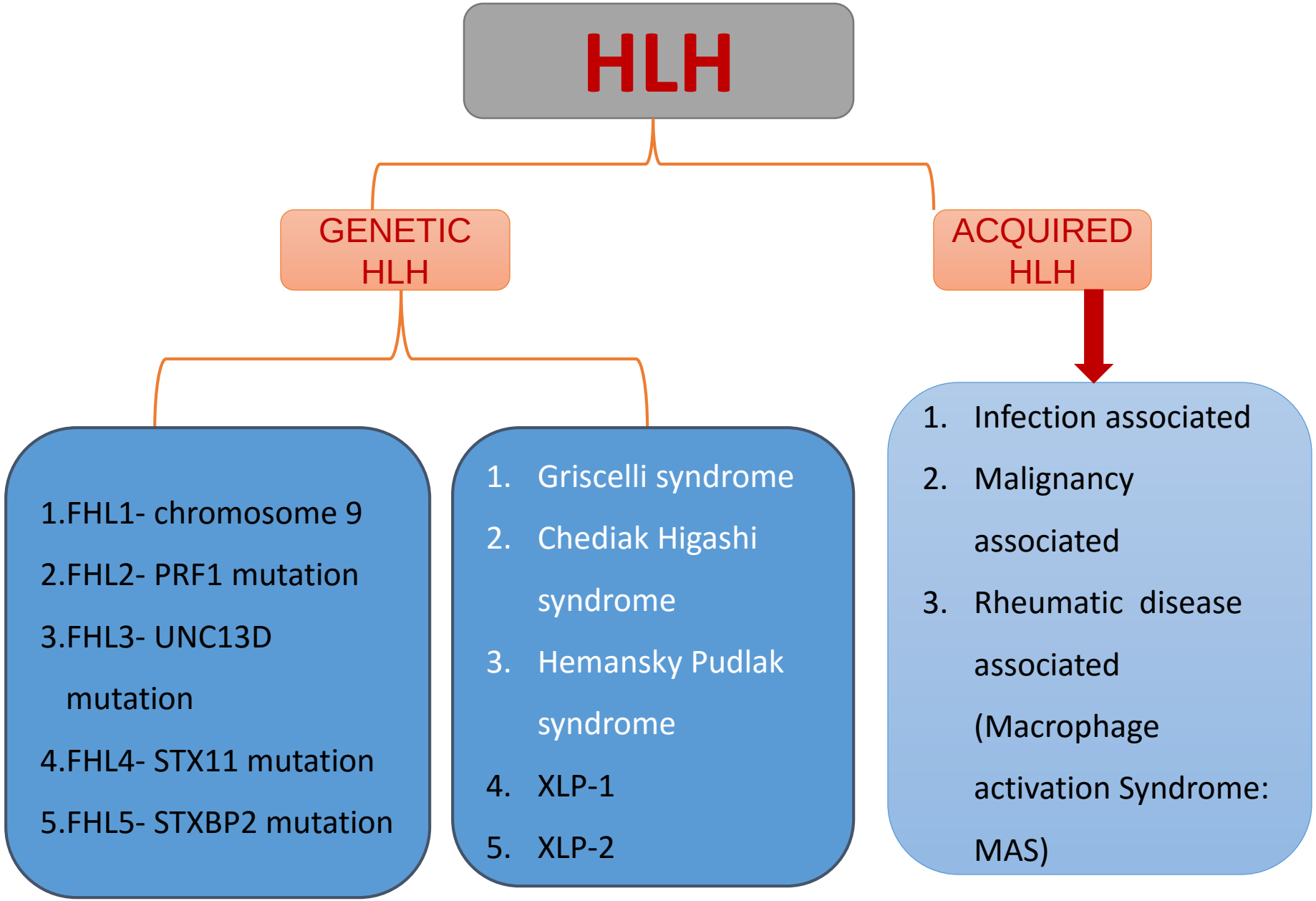
ACQUIRED HLH



Process	Protein
Sorting	LYST
Polarization	AP3B1
Docking	Rab27a
Priming	Munc13-4
Fusion	Syntaxin11, Syntaxin binding protein 2
Apoptosis	Perforin

- Malignancy
- Rheumatoid arthritis
- Viral infections
- Bacterial infections

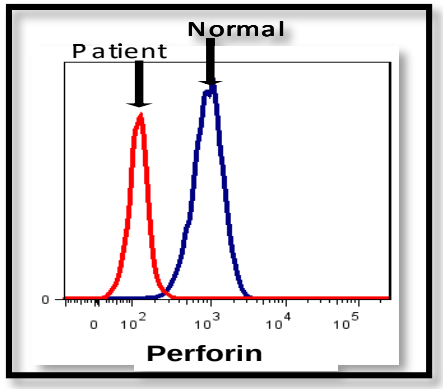

IFN-γ
• Cytopenia,Hemophagocytosis
• Procoagulant, Fever
• Low NK cell activity
IL-1β
• Lymphocyte infilteration, Fever
• Cytopenia, Hepatic cytolysis
• Hyperfibrinogenemia, Neurological disorders
IL-6
• Renal failure
• Fever
TNF-α
• Fever, Cytopenia
• Hypertriglygeridemia, Low NK cell activity
• Lymphocytic infilterartion


HLH
GENETIC HLH
ACQUIRED HLH
1.FHL1- chromosome 9
2.FHL2- PRF1 mutation
3.FHL3- UNC13D mutation
4.FHL4- STX11 mutation
5.FHL5- STXBP2 mutation
1. Griscelli syndrome
2. Chediak Higashi syndrome
3. Hemansky Pudlak syndrome
4. XLP-1
5. XLP-2
1. Infection associated
2. Malignancy associated
3. Rheumatic disease associated (Macrophage activation Syndrome: MAS)

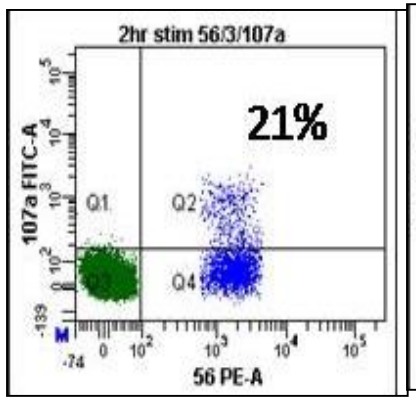
NK cell function evaluation:

Flow cytometry based tests

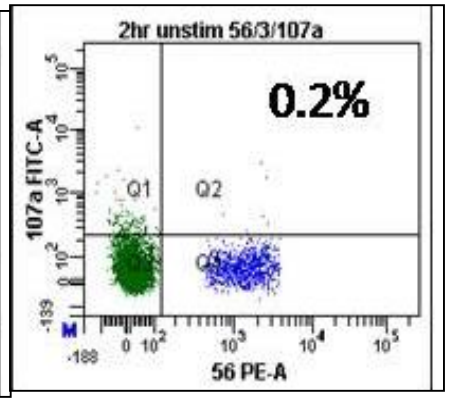
Perforin Expression



Granule Release Assay

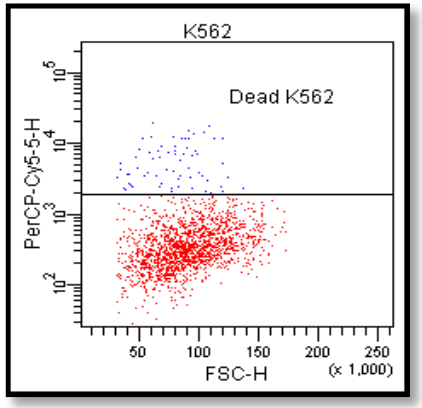


Control



FHL3/FHL4/ FHL5/GS

NK cell activity



- An 8-year-old boy was diagnosed with the Hodgkin lymphoma stage IVB based on lymph node and bone marrow biopsy reports. He received 6 cycles of ABVD chemotherapy, which contains the drugs adriamycin, bleomycin, vinblastine, and dacarbazine over a period of 5 months

- His health improved and was in remission when he presented with persistent high-grade fever, hepatosplenomegaly and thrombocytopenia.

- The bone marrow aspirate performed for persistent cytopenia at this stage showed hemophagocytosis, which prompted the work-up for HLH. He later presented with fungal sinusitis (Aspergillus infection) causing loss of vision in one eye.

- A 2-year-old boy presented with fever of unknown origin for >6 months. He had hepatosplenomegaly, anemia, neutropenia, and lymphocytosis.

- Peripheral smear showed atypical lymphocytes. He was suspected to have B-cell acute lymphocytic leukemia.

- His peripheral blood was also positive for CMV PCR. Bone marrow aspirate revealed hemophagocytes and hence was evaluated for HLH.

- At the age of 2 years presented with high grade fever, pancytopenia and hepatosplenomegaly. Treated as secondary HLH with steroids
- After tapering steroids developed relapse and hence cyclosporin was added
- She responded well and both steroids and cyclosporin tapered off
- Asymptomatic for nearly 6 months and then developed isolated neurological manifestations without cytopenia or elevated Ferritin
- A 4-year-old female presented with progreesive weakness on one side of the body

Unusual Clinical Presentations of Familial Hemophagocytic Lymphohistiocytosis Type-2

Snehal Mhatre, MSc, Manisha Madkaikar, MD,* Farah Jijina, MD,† and Kanjaksha Ghosh, MD**

Patients	2009-till date	In last 1 year 2018
Total Screened	698	90
FHL2-Perforin deficiency	39	3
GRA abnormal (FHL-3/FHL-4/FHL5)	37	8
Griscelli syndrome	11	0
Chediak- Higashi syndrome	5	0
Hermansky- Pudlak syndrome	1	0
XIAP	3	0
Total genetic HLH	**96**	**11**

Case 2

- 15 year old female
- High grade fever for 4 days and abdominal pain
- CBC revealed pancytopenia
- Elevated liver enzymes
- Serum ferritin level done at day 2 of admission was 56,000 ng/ml
- Serum procalcitonin level at admission was 2 ng/dl (RV: <0.5 ng/ml) which was indicative of bacterial sepsis.
- Serum triglyceride and plasma fibrinogen levels were within normal range.

- Ultrasound imaging showed mild splenomegaly, minimal free fluid in abdomen and edematous pancreas and bilateral pleural effusion.
- Work up of locally common infectious causes were done including urine and blood cultures, Epstein Barr Virus (EBV) Viral Capsid Antigen IgM, Cytomegalovirus (CMV) IgM, Malaria smear and antigen detection, Dengue NS1 antigen detection, Leptospira IgM serology, Weil Felix screening test for rickettsial infections.
- The Dengue NS1 antigen test was positive
- A diagnosis of dengue fever complicated by pancreatitis and hepatitis with associated chest infection was made.

- Despite supportive therapy she remained unwell with spikes of temperature, worsening abdominal pain and progressive bi-basal crepitations. Laboratory investigations showed persistent leukopenia, thrombocytopenia and rising aminotransferases.
- A bone marrow aspiration and trephine biopsy was done for further evaluation on day 4 of admission triggered by high ferritin and bicytopenia. Bone marrow study showed normocellular reactive marrow with evidence of macrophage activation and hemophagocytosis
- A dengue serology on day 6 of fever was positive for IgM antibodies but negative for IgG antibodies establishing the diagnosis of primary dengue infection with secondary HLH.

- the patient was given intravenous immunoglobulin (400 mg/kg/day for 5 days) starting on day 5 of hospitalisation in view of associated pancreatitis and chest infection.
- The patient continued to be febrile (T max 38.3 °C) till day 8 of admission. He improved over the next few days, became afebrile and was discharged after 13 days in the hospital. At the time of discharge his blood counts and biochemical parameters had recovered to almost normal levels.

Dengue and HLH

- A mild disease in majority of cases, less than 2% of dengue patients present with severe manifestations, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), both conditions are associated with considerable mortality and morbidity
- Secondary infection by a different serotype may predispose to DHF and DSS
- However DHF and DSS occur in first episode dengue infections also and HLH may have a role in these cases.
- Majority of the cases pulse dosage of methylprednisolone or dexamethasone have been used to suppress the hyperinflammatory state. Intravenous immunoglobulin G has been used in few cases either alone or with dexamethasone or methylprednisolone.

Virus induced HLH

HLH trigger	Organism	Cases	
Viral Infections	EBV	9	27
	CMV	6	
	Dengue	8	
	Adenovirus	4	
Bacterial infections	Typhus	1	1
Malignancy			4
MAS			2

FANCE (+) DDX58	
-	
UNC13D (-)	
FANCD2 (+)	
HMOX1	
MYSM1 (-) STXBP2 (+)	
No mutation	
NBN CR2	
DNAJ21	

CFHR3	CFHR1
CFHR3	CFHR1
MYSM1(-)	
PRF1	
UNC13D(-)	
BLOC1S6	

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Regular Article

PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Genetic and mechanistic diversity in pediatric hemophagocytic lymphohistiocytosis

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- Of 122 subjects enrolled over the course of 17 years, 101 subjects received genetic testing.
- Biallelic familial HLH (fHLH) gene defects were identified in only 19 (19%) and correlated with presentation at younger than 1 year of age ($P < .0001$).
- Digenic fHLH variants were observed but lacked statistical support for disease association.
- In 28 (58%) of 48 subjects, research whole-exome sequencing analyses successfully identified likely molecular explanations, including underlying primary immunodeficiency diseases, dysregulated immune activation and proliferation disorders, and potentially novel genetic conditions.

- Two-thirds of patients identified by the HLH-2004 criteria had underlying etiologies for HLH, including genetic defects, autoimmunity, and malignancy.
- In most patients with HLH, targeted sequencing of fHLH genes remains insufficient for identifying pathogenic mechanisms.
- Whole-exome sequencing, however, may identify specific therapeutic opportunities and affect hematopoietic stem cell transplantation options for these patients.

Case

- A 9-year-old girl presented a three-month history of abdominal pain, arthralgia, recurrent painless oral ulcers, bruises, epistaxis, anorexia, weight loss, night sweats, and fever.
- These complaints were preceded by agitation, aggression, and occipital headache that led her parents to ask for a psychiatric consult.
- She had no relevant past medical or travel history and no family history of rheumatic diseases.

- On physical examination, she had normal vital signs except for a temperature of 40°C.
- There was bilateral neck lymphadenopathy (1 × 1.5 cm) with splenomegaly and hepatomegaly.
- Laboratory studies:
- Pancytopenia: leukopenia (3600 cells/µL), normocytic anemia (7.8 g/dl), thrombocytopenia (78,000 cells/µL)
- hyperferritinemia (591 µg/L),
- hypertriglyceridemia (281 mg/dl),
- hyperfibrinogenemia (559 mg/dl), and increased levels of lactate dehydrogenase (606 IU/L).
- Coagulation profile normal.

- Liver and renal function normal
- Serological tests were negative for CMV, HBV, HCV, EBV, and HIV
- blood culture were also negative
- Immunologically, the patient was positive for ANA (1/320 homogenous), anti-dsDNA (240 IU/ml), and anti-Sm (91 IU/ml), and her serum C3 and C4 complement factors were low (33 mg/dl and 1.8 mg/dl, resp.)
- DCT/ICT positive
- Ultrasound revealed a moderate hepatosplenomegaly, and bone marrow aspiration revealed hyperactivity with some morphologically benign macrophages with an evidence of hemophagocytosis

Macrophage activation syndrome

- Macrophage activation syndrome (MAS) is a life-threatening complication of rheumatic diseases, requiring immediate and appropriate treatment.
- The most common autoimmune diseases associated with MAS are systemic juvenile idiopathic arthritis (SJIA), followed by systemic lupus erythematosus (SLE), Kawasaki disease (KD), and juvenile dermatomyositis (JDM)
- The symptoms of MAS are quite similar to those of many active autoimmune diseases or severe sepsis; therefore, it is quite difficult to make a diagnosis.

Macrophage activation syndrome

- The classical signs and symptoms of patients with MAS are a persistent high-grade fever, hepatosplenomegaly, lymph-adenopathy, and hemorrhagic manifestations.
- Abnormal results of investigation include cytopenia, coagulopathy, and hyperferritinemia.
- Approximately 35% of the patients with MAS developed a central nervous system (CNS) dysfunction, including seizures, as well as alterations in their mental status, although not necessarily at the same time. They also showed a certain amount of irritability, with lethargy, comas, and headaches

Conclude:

- HLH diagnosis requires high index of suspicion
- All the patients may not fulfil the criteria of HLH at the time of evaluation but may have progressive drop in counts
- All patients should be evaluated for underlying fHLH irrespective of age or identification of a secondary cause
- Extensive genetic testing especially whole exome analysis is recommended but its impact on management is still not clear